ULTRAVIOLET LIGHT INDUCED RECOVERY IN *ESCHERICHIA COLI* OF RADIATION DAMAGED BACTERIOPHAGE DEOXYRIBONUCLEIC ACID

By

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The ability of ultraviolet light (UV) ØX174 single stranded and replicative form DNA molecules to produce whole phage when transfected into UV irradiated, calcium treated *E. coli* K12 hosts was investigated. When UV irradiated single and replicative form ØX174 DNA molecules were transfected into UV irradiated wild type hosts, an enhanced survival of the phage producing ability of the DNA was observed over that seen when the transfection was performed with unirradiated wild type hosts. A similar increase in survival was also found for both types of ØX174 DNA molecules when they were transfected into *E. coli* deficient in excision repair (*uvr*A-, *uvr*B- or *uvr*C-) or recombinational repair (*rec*B- or *rec*B- *rec*C-). No enhanced survival was found with *rec*A- or *lex*A- *E. coli* hosts. The level of the increased phage producing ability for single stranded ØX174 DNA was 1.3 to 1.7 times greater than that for the replicative form DNA in all genetic backgrounds which showed enhanced survival.

These results suggest the existence of a UV inducible recovery system which participates in the recovery of UV irradiated ØX174 DNA.

This recovery system is dependent upon recA and lexA regulated functions but is independent of excision and recombinational repair. The common genetic requirements for the increased survival of both forms of ØX174 DNA suggest a common mechanism of recovery for both. The greater survival of the single stranded molecule indicates that it is more susceptible to inducible recovery than the replicative form molecule which may be due to the physical differences between the two.

The ability of gamma irradiated ØX174 single stranded and replicative form DNA to produce whole phage when transfected into a UV irradiated, calcium treated E. coli wild type host was also investigated. By adding appropriate radical scavengers to aqueous solutions of the DNA, it was possible to specify which of the water radiolysis radicals was interacting with the DNA. The maximal enhancement of phage producing ability for both types of DNA was observed under conditions which removed the hydroxyl radical allowing the hydrogen radical and the solvated electron to predominate. Scavenging conditions which removed the hydrogen radical and solvated electron as well as the hydroxyl radical resulted in an enhancement of survival only half as large as the maximum. The same was found to be true for scavenging conditions which removed the hydrogen radical and solvated electron only.

These results demonstrate the existence of a UV inducible repair system which mediates in the recovery of ØX174 DNA from gamma ray damage. The mechanism of recovery here could possibly be the same as that which affected the UV inducible recovery of UV irradiated ØX174 DNA. From these results, it can also be concluded that the hydrogen radical and the solvated electron produce a type of damage that is more susceptible to inducible recovery than the hydroxyl radical. The hydroxyl radical

appears to produce two classes of damage in both the single stranded and replicative form DNA molecules. The first is susceptible to inducible recovery while the second is refractory to it. This second class of damage may inhibit recovery from damage caused by the hydrogen radical and the solvated electron.

INTRODUCTION

The biological functions of deoxyribonucleic acid (DNA) are very sensitive to ultraviolet (UV) and ionizing radiation. These radiations produce physico-chemical alterations in DNA which may result in death or mutations. There are enzymatic repair systems which remove the biological damage. A brief discussion of radiation damage and its repair in the bacterium *Escherichia coli* follows. This will provide background information pertinent to this dissertation which is directed toward characterizing one of these repair systems.

UV Damage in DNA

UV radiation produces a variety of alterations in DNA molecules. Such damage includes cyclobutane dipyrimidine dimers (pyrimidine dimers or dimers), pyrimidine hydrates, pyrimidine adducts and DNA-protein cross-links (44, 77). Most of the lethality and mutagenesis caused by UV can be attributed to the presence of dimers rather than other photoproducts in DNA. The basis for this statement is the fact that photoreactivation, which acts specifically on pyrimidine dimers, can remove 50 to 90% of the dimers from bacterial DNA which results in a corresponding decrease in the biological effect of UV (44). Thus, in the following discussion of UV repair systems, only the repair of dimers will be considered.

Repair of Dimers

Photoreactivation

Photoreactivation results in the *in situ* splitting of pyrimidine dimers (68). The mechanism of this repair employs an enzyme which

binds specifically to pyrimidine dimers. When exposed to light (300-400 nm), the enzyme-dimer complex is activated and the dimer is split without breaking the phosphodiester backbone of the DNA molecule. In concept, photoreactivation should not be error prone which is confirmed by the observation that it is at least 90% error free (92).

Excision Repair

A second repair pathway can remove dimers in the absence of light. In E. coli excision repair breaks the phosphodiester backbone in the tide and then closes the resulting gap by de novo DNA synthesis (10, 75).

The incision step is absent in uvrA and uvrB mutants (40). These mutants lack E. coli correndonuclease II, an endonuclease which specifically makes a nick on the 5' side of pyrimidine dimers (11, 31). The nick with 5' phosphoryl and 3' hydroxyl termini can be sealed by polynucleotide ligase. Premature closure of this nick, which aborts excision repair is observed in uvrC mutants (13, 47). Also, uvrC lig^{ts} mutants contain more nicks in their DNA after UV irradiation than uvrC single mutants (74). These results suggest that the uvrC gene product protects the incision from being resealed by ligase.

In wild type E. coli, excision of the dimer is carried out by DNA polymerase I (19, 30, 48). Beginning at the 5' phosphoryl end of the nick, DNA polymerase I, acting as a 5' to 3' exonuclease, degrades a short segment (approximately 30 nucleotides) of the DNA strand containing the dimer as oligonucleotides. The gap resulting from this excision is filled concurrently by DNA polymerase I using the complementary strand as a template. In the absence of DNA polymerase I, DNA polymerase II or III can perform the excision-resynthesis step (55, 82).

The final step of excision repair is the rejoining of the phosphodiester backbone between the preexisting, undamaged DNA strand and the newly synthesized DNA by ligase.

An alternative type of excision repair termed "long patch" (as opposed to "short patch" described above) has been found in polA mutants (31). The excision step of long patch repair removes at least 100 times as many nucleotides as the short repair excision step. Long patch repair is growth medium dependent, and is inhibited by chloramphenicol. It requires the uvrA, recA, recB, lexA and polC gene products (95). The common requirement for uvrA$^+$ suggest that long patch repair and short patch repair share the same incision step but differ in their excision-resynthesis steps.

Replication on Damaged Templates

Bacterial DNA synthesized immediately after UV irradiation is discontinuous (67, 76). The post-irradiation molecular weight of the newly synthesized DNA approximates the value calculated for the inter-dimer distance after a given UV dose. It has been proposed that dimers block the progress of bacterial DNA replication (67). They do not inhibit subsequent reinitiation beyond the dimer at a new initiation site. Thus, it is possible that gaps up to 1,000 nucleotides long may be left in the newly synthesized DNA after irradiation (43). These gaps have 5' phosphoryl and 3' hydroxyl termini. They can be closed by DNA polymerase I and ligase but only after the dimer has been removed by photoreactivation. This suggests that dimers can block the polymerizing activity of DNA polymerase I (12).

Post-replication Repair

The daughter strand gaps caused by replication of UV damaged DNA do not persist indefinitely. Instead, the gaps are filled and sealed so that the molecular weight of the newly synthesized DNA becomes the same as that of DNA synthesized on unirradiated template. This process is called post-replication repair (67).

Post-replication repair occurs in E. coli by a variety of pathways (65, 72, 97). All post-replication repair pathways are recA dependent which suggests a recombinational mechanism (42, 76). Both the recB, recC and recF recombination pathways have been shown to contribute to post-replication repair (65, 97). recF mutants show a greater inhibition of post-replication repair than recB recC mutants, even though both have the same sensitivity to UV (65). This indicates that the recB recC product, exonuclease V, contributes to recovery from UV predominantly by some action unrelated to post-replication repair.

There are at least two post-replication repair pathways which are controlled by gene products that are not involved in recombination. One is dependent upon lexA while the other requires uvrD (29, 72, 96, 97). Apparently these and the recombination dependent pathways are arranged into a recA dependent, multi-branched post-replication repair pathway in wild type E. coli (72, 97).

Inducible Recovery of UV Damage

SOS Hypothesis

In E. coli there are a number of UV inducible phenomena which contribute to the recovery of UV damage. These include:

1. W-reactivation of UV irradiated bacteriophage (21, 22, 36, 49, 90).

2. Error prone repair mutagenesis of bacteria and their phage (6, 22, 71, 90, 93).
3. UV inducible cell division delay and filamentation (61).
4. Induction of a pathway of post-replication repair (13, 71, 73, 97).
5. Inhibition of post-irradiation DNA degradation (53).
6. Cessation of respiration following irradiation (79).

The induction of all of these phenomena is recA and lexA dependent and is sensitive to chloramphenicol indicating that de novo protein synthesis is required for their expression (94). A variety of agents other than UV such as ionizing radiation, thymine starvation, naladixic acid and non-permissive temperatures in dna B mutants will lead to induction (94). Finally, all of these phenomena occur in excision repair and post-replication repair deficient (other than recA or lexA) cells (94). This indicates that inducible recovery from UV damage proceeds by mechanisms unique from the constitutive repair systems.

The SOS hypothesis of Radman is an attempt to find a common basis for the participation of these phenomena in UV repair (60, 61). The hypothesis states that treatments which abruptly interrupt DNA replication triggers the coordinate expression and de novo synthesis of a set of gene products which function by various mechanisms in the repair of UV damage. The derepression of these gene products is jointly regulated by the recA and lexA genes. Radman also proposes that the molecular mechanism of some of the inducible repair phenomena involves an error prone DNA polymerase. The repair functions involving such a hypothetical polymerase are referred to as SOS repair.

SOS Repair

Biological evidence provided by experiments with single stranded phage such as ØX174 supports the error prone DNA polymerase mechanism of SOS repair. The heart of the biological evidence lies in the fact that during replication these phage must first convert their single stranded genome into a double stranded replicative form (50). UV induced dimers inhibit this conversion by blocking DNA replication at the point of the radiation lesion and prevent the reinitiation of replication beyond it (54, 62, 63). This renders the DNA molecule biologically inactive. Increased survival with a concomitant increase in mutagenesis (W-reactivation) has been observed when UV irradiated single stranded bacteriophage infect wild type E. coli host which have also been UV irradiated so that they express SOS repair (6, 21, 58, 83). This can only occur if the damaged single stranded genome is converted into its double stranded form.

There is also biochemical evidence supporting the error prone DNA polymerase mechanism of SOS repair. When UV irradiated ØX174 DNA or oligodeoxyadenylic acid primed polydeoxythymidylic acid have been used as templates for DNA synthesis in a crude extract of UV irradiated E. coli, both are converted to full length, intact, double stranded forms (62). A high level of misincorporation of nucleotides also has been observed in the experiments utilizing the polydeoxythymidylic acid as a template (62).

From this Radman deduces the error prone DNA polymerase mechanism for SOS repair. To account for these observations, the UV induced polymerase must be able to polymerize DNA on a damaged template. Misincorporation is presumably caused by the damaged nucleotides in the template either being non-instructive or coding for a non-complementary

nucleotide (60, 61, 62). Alternative explanations suggest that misincorporation may be an inherent property of the induced polymerase itself. The polymerase may be faulty in selecting complementary nucleotides or it may be defective in a proof reading exonuclease function similar to that of DNA polymerase I which can excise a mismatched nucleotide from a newly synthesized DNA strand (50, 52). These alternative explanations are supported by the observation of a mutator effect of SOS repair on unirradiated DNA (94).

Treatments which elicit the inducible repair phenomena to date have not been shown to result in the appearance of a new polymerase with the properties necessary to accomodate the SOS repair mechanism (94). If it is assumed that SOS repair includes W-reactivation of double stranded bacteriophage and error prone repair mutagenesis of bacteria, some speculations can be made as to the involvement of DNA polymerases I and III in SOS repair. Polymerase I can be eliminated as a participant since polA mutants support W-reactivation of lambda phage and UV induced bacterial mutagenesis (15, 93). It has been shown, however, that $dnaE^{ts}$ mutants do not show UV induced mutagenesis at non-permissive temperatures. This implicates DNA polymerase III as having a role in SOS repair (13). One speculation is that one of the inducible recovery phenomena is the synthesis of a modifying factor which causes DNA polymerase III to become an error prone DNA polymerase (94).

Gamma Ray Action on DNA

Gamma rays interact with aqueous solutions of DNA molecules in two manners. In the first, gamma rays cause ionizations directly in the DNA molecule resulting in physico-chemical alteration. This manner of

interaction is called direct action (24). The second mode of interaction is called indirect action (24). The gamma rays in this case interact with the water molecules of the solution causing them to become excited or ionized. By a series of secondary reactions, the excited or ionized water molecules break down to form a set of free radicals, the water radiolysis radicals. The primary water radiolysis radicals are the hydrogen radical, the solvated electron and the hydroxyl radical (26). All of these radicals are very reactive with DNA causing either electron additions or abstractions which can alter the molecule (9, 18).

Use of Scavengers in the Study of Indirect Action

It is possible to remove one or more of the water radiolysis radicals from a gamma irradiated aqueous solution of DNA molecules by the use of radical scavengers. The best scavengers are chemical species which have high bimolecular rate constants for their reaction with a given radical and form reaction products which are innocuous to DNA. Thus, certain radiolysis radicals may be prevented from reacting with the DNA so that the effects of the remaining ones may be more clearly delineated.

By using the appropriate radical scavengers, it has been possible to determine the contribution of the water radiolysis radicals to the biological inactivation of DNA. Work with the single stranded DNA molecule of ØX174 has indicated that at least 95% of the gamma ray inactivation of its biological activity is due to hydroxyl radicals (8). Other experiments with the same molecule have suggested that the solvated electron does not contribute at all to inactivation (9). A large increase in the survival of the biological activity of ØX174 replicative form DNA is seen when it is gamma irradiated under conditions which effectively

scavenge the hydroxyl radical while a much smaller increase in protection is seen when the hydrogen radical and the solvated electron are scavenged (2). All of these observations support the contention that the hydroxyl radical is the major radical species contributing to the biological inactivation of DNA in aqueous solution.

Gamma Ray Induced Damage in DNA

Gamma ray damage may be put into two broad groups; strand breaks (single and double) and base damage. A strand break is a disruption of the phosphodiester backbone of the DNA molecule. *In vitro* studies with aqueous solutions of bacteriophage DNA molecules have shown that the hydroxyl radical is almost exclusively responsible for producing strand breaks (2). Strand breaks are detected in the DNA gamma irradiated *E. coli*, but the complex chemical environment surrounding cellular DNA makes it impossible to attribute the production of strand breaks in this instance to any one of the water radiolysis radicals.

In DNA all four of the purine and pyrimidine bases can be altered by gamma irradiation (18, 38, 69). Unfortunately, the doses required to produce these alterations are far greater than those needed to produce a physiological effect (9). There are, however, two types of base damage in irradiated DNA molecules which can be detected in the physiological dose range. The first are alterations of thymine which produce a class of damage products of the 5, 6 dihydroxyl-dihydrothymine type (17, 18). The other type of base damage is that which produces apurinic and apyrimidinic sites in DNA (18, 51). The hydroxyl radical has been implicated in the formation of the 5, 6 hydroxyl-dihydrothymine type of damage (64, 66).

Some indication of the relative contribution to lethality of the two types of gamma ray damage has been determined from experiments with bacteriophage DNA (9, 18). Almost 90% of lethality in gamma irradiated ØX174 replicative form DNA and PM2 DNA has been attributed to base damage (87). On the other hand, 50 to 95% of the biological inactivation of ØX174 single strand DNA is due to strand breaks (7, 9). Thus, double stranded DNA is more susceptible to biological inactivation by base damage than single stranded DNA even though the efficiency of inducing base damage in single and double stranded DNA is very similar (18). The chemical nature of the lethal base damage is not known and the role of base damage in the *in vivo* inactivation of living cells has yet to be determined (18).

Repair of Gamma Ray Damage

Strand Break Repair

The repair of single strand breaks in *E. coli* appears to proceed by several mechanisms dependent upon the chemical nature of the break and post-irradiation conditions. It has been suggested that the repair of single strand breaks produced under anoxic conditions may be mediated by polynucleotide ligase (84, 85, 86). Under aerobic conditions most strand breaks produced in *polA*$^+$ *E. coli* are repaired in less than one minute after irradiation while *polA* mutants show little repair under the same conditions (84). This type of DNA polymerase I dependent strand break repair is absent if the irradiation is done under anoxic conditions. Breaks generated under these conditions are closed very slowly and only during incubation at 37° C in the presence of nutrients. This slow type of strand break repair is missing in recombination deficient *E. coli* (46, 84).

In E. coli no repair of double strand breaks has been found, and the killing efficiency of such damage is assumed to be 100% (45).

Repair of Base Damage

Removal of the 5, 6 dihydroxyl-dihydrothymine type of base damage has been carried out in vitro using crude extracts of E. coli (17). The involvement of the 5' to 3' exonuclease activity of DNA polymerase I in the removal of the damage and of polynucleotide ligase in the last sealing step has been demonstrated (33, 34). The uvrA uvrB endonuclease, correndonuclease II, has been shown not to be involved in the repair of this damage (16, 35). An endonuclease has been discovered in E. coli which specifically acts on X-irradiated DNA (78). Whether or not this endonuclease plays a role in the repair of 5, 6 dihydroxyl-dihydrothymine type of base damage has not been established.

An endonuclease which hydrolyzes the phosphodiester bond near apurinic sites has been found in E. coli (88, 89). This hydrolysis represents the first step in the repair of such sites. The in vitro incubation of DNA with this endonuclease along with DNA polymerase I and polynucleotide ligase results in the removal of apurinic sites (88).

Inducible Recovery of Gamma Ray DNA Damage

There are several reports of SOS type repair of X or gamma ray damaged DNA. X-irradiated ØR, a single stranded bacteriophage showed W-reactivation when infected into UV or X-irradiated E. coli hosts (58). However, similar experiments with X-irradiated lambda phage resulted in no W-reactivation (36). Increased survival of the colony forming ability of gamma irradiated E. coli which had previously received a smaller UV or gamma ray dose has been reported (59). This indicates that SOS repair plays a role in the repair of gamma ray damaged cellular DNA.

Object of this Study and Experimental Approach

The objective of this dissertation is to characterize further the UV induced recovery in E. coli of radiation damaged bacteriophage DNA. The specific goals of this study are:

1. Comparison of the efficiency of UV induced recovery of UV irradiated single and double stranded DNA.
2. Determination of the relationship of UV induced recovery of UV irradiated single and double stranded DNA to excision repair and recombination.
3. Determination of the role of the various gamma ray water radiolysis radicals in producing damage in single and double stranded DNA susceptible to UV inducible recovery.
4. Comparison of the UV induced recovery of UV irradiated versus gamma irradiated DNA molecules.
5. Evaluation of whether or not the proposed mechanism of SOS repair will explain the UV induced recovery of both single and double stranded DNA.

The experimental approach to meeting these objectives employs a purified ØX174 DNA-calcium treated E. coli transfection system. Calcium treated E. coli hosts which have or have not been induced by UV irradiation are transfected with UV or gamma irradiated ØX174 single stranded and replicative form DNA. The ability of the transfected, irradiated DNA molecules to produce whole phage is then measured by an infective center plaque assay. Inducible recovery is indicated by a greater survival of phage producing ability when the irradiated DNA is transfected into UV irradiated hosts as opposed to unirradiated hosts.

MATERIALS AND METHODS

Bacterial Strains

The genotypes for all strains and their references appear in Table I. All strains are *E. coli* K12 and were derived from AB1157 with the exception of HF4714 which is a C strain *E. coli*. The nomenclature is that proposed by Demerec *et al* (23). The relevant genetic markers affecting radiation repair were checked by UV sensitivity.

Bacterial Growth Conditions

All cells were grown at 37° C with aeration in medium BX (8 gm KCl, 0.5 gm NaCl, 1.1 gm NH_4Cl, 0.8 gm sodium pyruvate, 23 mg KH_2PO_4, 20 mg $MgCl_2$, 0.11 gm $CaCl_2$, 25 mg Na_2SO_4, 11.44 gm Trizma-HCl, 3.32 gm Trizma base, 10 mg uracil, 2 mg thymine, 15 gm Bacto casamino acids - vitamin free, 20 mg tryptophan, 20 mg thiamine and 4 gm glucose per liter of distilled water). Culture growth was followed using a Klett-Summerson colorimeter with a blue filter.

ØX174 DNA Preparation

Single stranded ØX174 DNA was prepared by two phenol extractions at room temperature of bacteriophage ØX174 am3, a lysis defective mutant (14). The double stranded, replicative form ØX174 DNA was prepared by using the method previously described by Achey *et al*. with the exception that the column step was replaced by dialysis (1). Both DNA preparations were extensively dialyzed against 0.001 M sodium phosphate buffer, pH 7.2 or 0.05 M Tris, pH 7.2 as a last step in their preparation.

TABLE I

BACTERIAL STRAINS

Strain	Genotype	Reference
AB1157	argE3, his-4, leu6 proA2, thr-1, str-31, galK2, lacY1, xyl5, mtl-1, ara-14, tsx-33, supE44, thi-1	3
AB1884	uvrC34	40
AB1885	uvrB5	40
AB1886	uvrA6	40
AB1889	uvrA35	40
AB2463	recA13	91
AB2470	recB21	91
AB2494	lexA1	41
JC5519	recB21 recC22	91
HF4714	thy uvrA supX	4

ØX174 DNA UV Irradiations

The UV source was three 15 watt General Electric L15T8 germicidal bulbs. The intensity of the bulbs could be regulated by altering the voltage to them with a rheostat. Two dose rates were used to irradiate the DNA. A rate of 1.0 $J/m^2/s$ was used for delivering doses less than 100 J/m^2. Higher doses were delivered at a rate of 10 $J/m^2/s$. Dose rates were determined with an International Light Germicidal/Erythemal Radiometer model IL570.

A 0.1 ml sample of the single and double stranded DNA in 0.05 M Tris, pH 7.2 at a concentration of 5.0 μg/ml were placed in a 7 cm watch glass and exposed to a series of increasing UV doses while being stirred. After each dose, 10 μl aliquots of the irradiated DNA were diluted 100 fold into 0.05 M Tris, pH 7.2. The diluted DNA was kept on ice in anticipation of the biological assay.

ØX174 DNA Gamma Irradiations

Gamma irradiations were carried out in a custom built cobalt-60 gamma irradiator (32). Sample holders were positioned in the source so that dose rates of 4.7 rads/s and 185 rads/s were available. The dose rates were determined by ferrous-ferric sulfate dosimetry.

The DNA concentration at irradiation was 5.0 μg/ml and the DNA was in 0.001 M sodium phosphate buffer during irradiation. This buffer was chosen since it does not appreciably affect the radiosensitivity of the DNA in solution (1). The scavengers used in these experiments, the radicals with which they react, the reaction bimolecular rate constants and the reaction products formed are listed in Table II. None of the scavenger-radical reaction products formed react with DNA (9, 95). All of the samples (0.2 ml volume) were flushed immediately

TABLE II

BIMOLECULAR RATE CONSTANTS OF THE PRIMARY WATER RADIOLYSIS RADICALS WITH THE RADICAL SCAVENGERS USED IN THIS STUDY

Scavenger	Bimolecular Rate Constant (M^{-1} sec^{-1})*		
	Hydroxyl Radical	Hydrogen Radical	Solvated Electron
N_2	--	--	--
O_2	--	2.6×10^{10}	1.9×10^{10}
I^-	7×10^9	--	--

*See reference 26.

prior to irradiation with either water saturated nitrogen or oxygen. The samples were bubbled for at least 5 minutes at a flow rate of 60 cc/min to assure saturation with the gas. The concentration of potassium iodide was 0.05 M whenever it was used as a scavenger. This concentration ensures maximal scavenging of the hydroxyl radical (9).

Samples of the irradiated DNA solution were diluted 100 fold immediately into 0.05 M Tris, pH 7.2. The diluted DNA was kept on ice in anticipation of the biological assay.

UV Irradiation and Induction of E. coli

E. coli were grown in medium BX to a density of 1.7×10^8 cells/ml (Klett=100). 25 ml aliquots of this culture were centrifuged at 10,000 rpm for 5 minutes to pellet the cells. The supernatant was discarded and the pelleted bacteria were resuspended in 25 ml of phage buffer (7 gm Na_2HPO_4, 3 gm KH_2PO_4, 5 gm NaCl, 0.12 gm $MgSO_4$, 0.01 gm $CaCl_2$ and 0.01 gm gelatin per liter of distilled water). The resuspended bacteria were then poured into a 12 cm diameter, flat-bottomed dish and exposed to UV while being vigorously stirred. For total doses greater than 10 J/m^2 a dose rate of 0.1 J/m^2 was used. The irradiated culture was then centrifuged as before, the supernatant discarded and the pellet resuspended in 25 ml of fresh medium BX. This culture was then incubated in the dark for 50 minutes at 37° C with aeration to permit expression of the inducible recovery system (59). The unirradiated control samples were treated identically with the exception that they received no UV irradiation.

Calcium Treatment of E. coli

The calcium treatment procedure is essentially that of Taketo (80). Immediately after the 50 minute post-irradiation incubation, 20 ml samples of the cultures were centrifuged as before and the supernatants discarded. The pellets were resuspended in 10 ml of an ice cold solution of 0.05 M $CaCl_2$, 0.005 M $MgCl_2$ and 2% polyethylene glycerol (57, 81). The resuspended bacteria were kept in an ice water bath for 30 minutes before being centrifuged and the supernatant discarded. This final pellet was resuspended in 1.0 ml of the 0.05 M $CaCl_2$, 0.05 M $MgCl_2$ and 2% polyethylene glycerol solution and then stored overnight at 0° C prior to being used to assay the biological activity of the ØX174 DNA.

All steps subsequent to the UV irradiation were performed in yellow lights to prevent photoreactivation.

Transfection and Biological Assay of ØX174 DNA

The transfection procedure is also basically that of Taketo (80). 100 µl of the diluted, irradiated DNA and 200 µl of the calcium treated bacteria were mixed together and incubated in an ice water bath for 30 minutes. The transfection mixture then was incubated at 37° C for three minutes before being returned to the ice water bath for an additional 5 minutes before plating.

The infective centers produced by the above procedure were detected by a plaque assay. This consisted of plating appropriately diluted samples of the transfection mixture with 10 ml of top agar (2.5 gm NaCl, 2.5 gm KCl, 10 gm Bacto-tryptone, 10 gm Bacto-agar and 6 ml of 1 N NaOH per liter of distilled H_2O) seeded with the indicator strain E. coli HF4714. The plates were incubated at 37° C for 4 to 6 hours.

All of the above steps were done in yellow lights to prevent photoreactivation.

Evaluation of UV Inducible Recovery Ability

The ability of UV inducible recovery to promote the survival of the phage producing ability of irradiated ØX174 DNA was determined by comparing the dose survival curves of the DNA in a UV induced host and an unirradiated host. As shown in Figures 1 and 2 using data from this study, UV induced recovery was indicated by the dose survival curves for the ØX174 DNA in the UV irradiated host having a shallower final slope than that for the unirradiated host.

Rather than comparing sets of dose survival curves, the level of UV induced recovery is best measured by the percent efficiency of repair which is determined by the expression:

$$\% E = (\log S_2 - \log S_1)/(\log S_2 - \log S_0) \times 100$$

(5, 25). As shown in Figures 1 and 2, So is the survival of the unirradiated ØX174 DNA molecule (fraction survival equals 1.0), S_1 is the survival of the ØX174 DNA irradiated with dose D when transfected into a UV induced host and S_2 is the survival of the ØX174 DNA at dose D when transfected into an unirradiated host. So, S_1 and S_2 have been determined from dose-survival curves which are the average of at least three independent experiments. The efficiency of recovery expresses the change in the final slope of the survival curve of the irradiated DNA from steeper to shallower in the UV induced host as the fraction of potentially lethal damage which is removed by UV induced reapir.

Figure 1. Dose survival curve of UV irradiated ØX174 single stranded DNA transfected into E. coli AB1157. Triangles, unirradiated E. coli AB1157 as host; circles, UV irradiated (70 J/m^2) E. coli AB1157 as host. The parameters necessary for determining the percent efficiency of repair are shown.

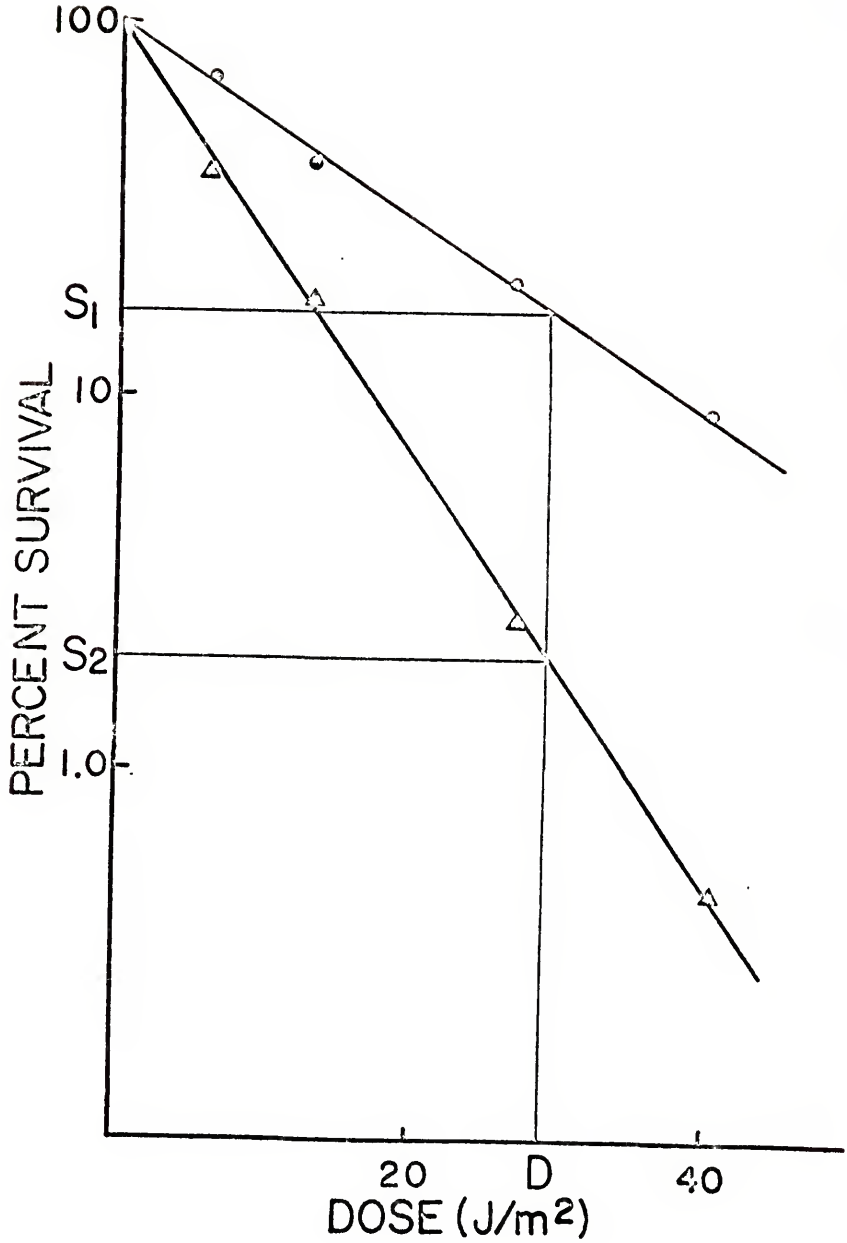
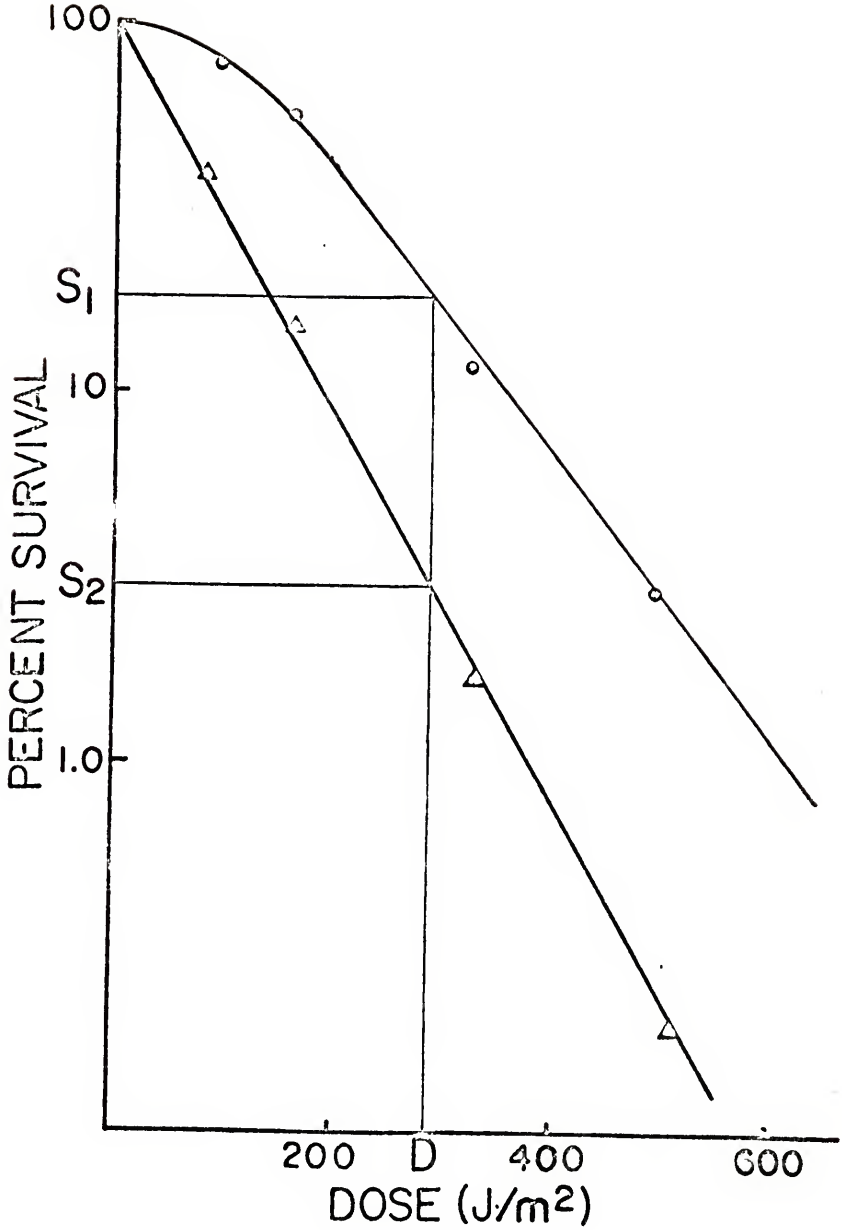

100
S1
10
S2
1.0
PERCENT SURVIVAL
20
D
40
DOSE (J/m2)

Figure 2. Dose survival curve of UV irradiated ØX174 replicative form DNA transfected in *E. coli* AB1157. Triangles, unirradiated *E. coli* AB1157 as host; circles, UV irradiated (70 J/m^2) *E. coli* as host. The parameters necessary for determining the percent efficiency of repair are shown.


100
S1
10
S2
1.0
PERCENT SURVIVAL
200
D
400
600
DOSE (J/m2)

RESULTS

UV Induced Recovery of UV Irradiated DNA

Figures 3 through 9 show dose induction kinetics plots of the efficiency of UV induced recovery of UV irradiated single stranded and replicative form ØX174 DNA in wild type, uvrA, uvrC, recB, recB recC, recA and lexA hosts. Such plots illustrate the change in the amplitude of the efficiency of UV induced recovery as the UV inducing dose to the host is varied.

The wild type, uvrA, uvrC, recB and recB recC host all show UV induced recovery of both the single stranded and replicative form ØX174 DNA while the recA and lexA hosts show no UV induced recovery. In all hosts showing UV induced recovery, the amount of recovery for both types of DNA molecules changes in a parallel fashion as the UV inducing dose is increased. In all cases, however, the efficiency of recovery is always 1.3 to 1.7 times higher for the single stranded DNA molecule than for the replicative form.

Table III lists the maximal efficiencies of UV induced recovery and the UV inducing doses that elicit them for the hosts which show UV induced recovery. The wild type strain AB1157 shows the greatest efficiency of recovery. At best, a single stranded ØX174 DNA molecule can recover from an additional 60% of the potentially lethal UV damage not repaired by other repair mechanisms while the replicative form DNA molecule can recover only from 45% of such damage. The other hosts show only 54% to 75% of the wild type efficiency of recovery of

Figure 3. Dose induction kinetics plot for E. coli AB1157. Circles, single stranded ØX174 DNA, triangles, replicative form ØX174 DNA.

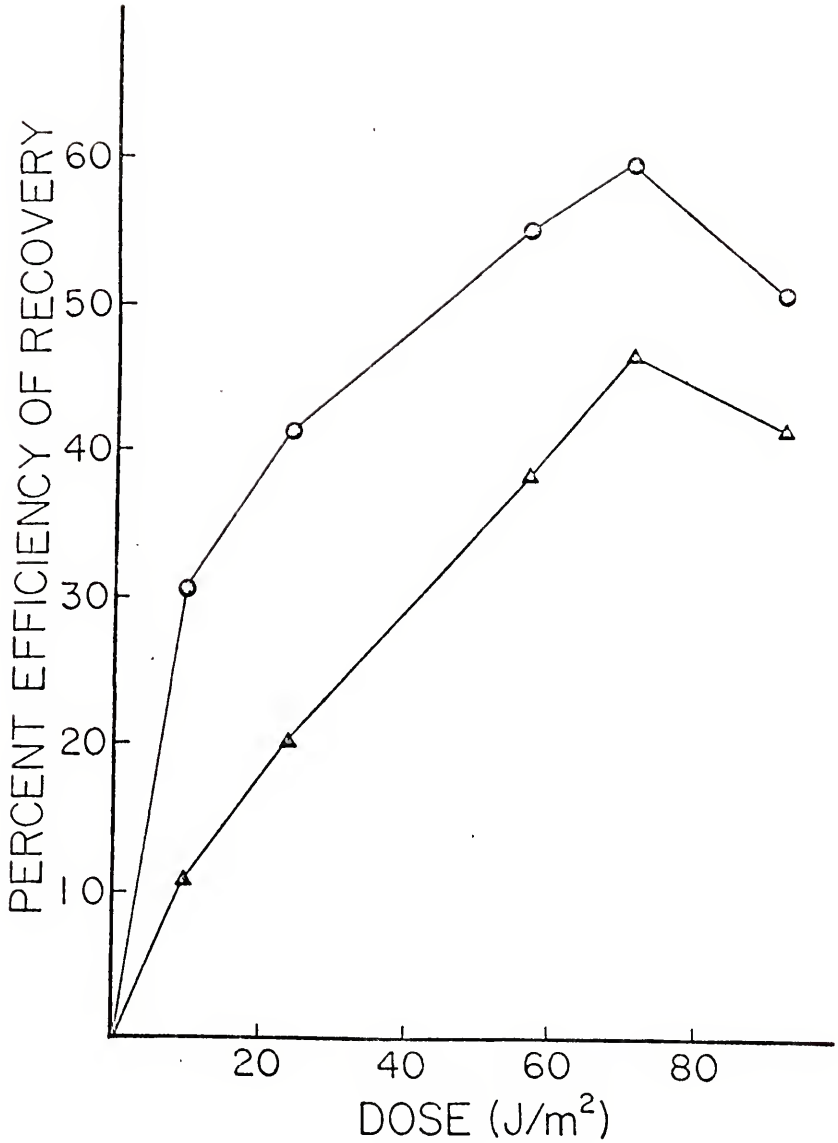

PERCENT EFFICIENCY OF RECOVERY
60
50
40
30
20
10
20
40
60
80
DOSE (J/m²)

Figure 4. Dose induction kinetics plot for *E. coli* AB1886 *uvrA*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

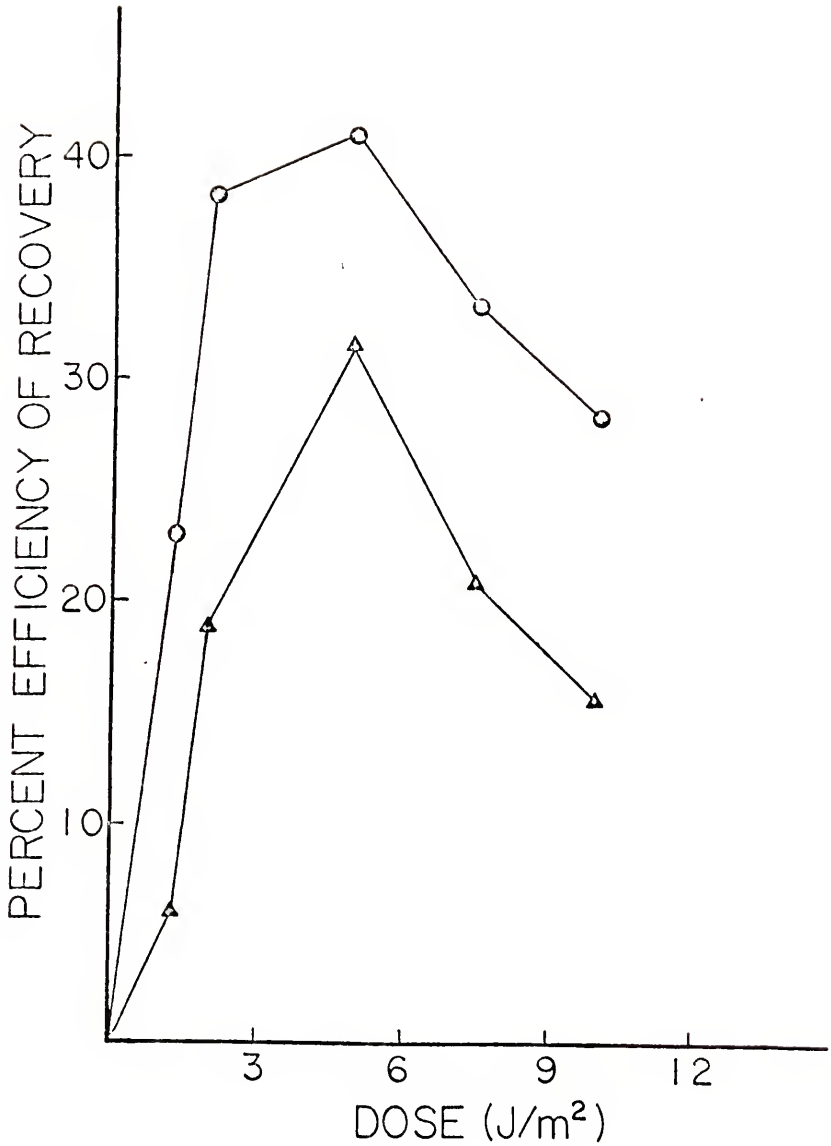

PERCENT EFFICIENCY OF RECOVERY
40
30
20
10
3
6
9
12
DOSE (J/m²)

Figure 5. Dose induction kinetics plot for *E. coli* AB1884 *uvrC*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

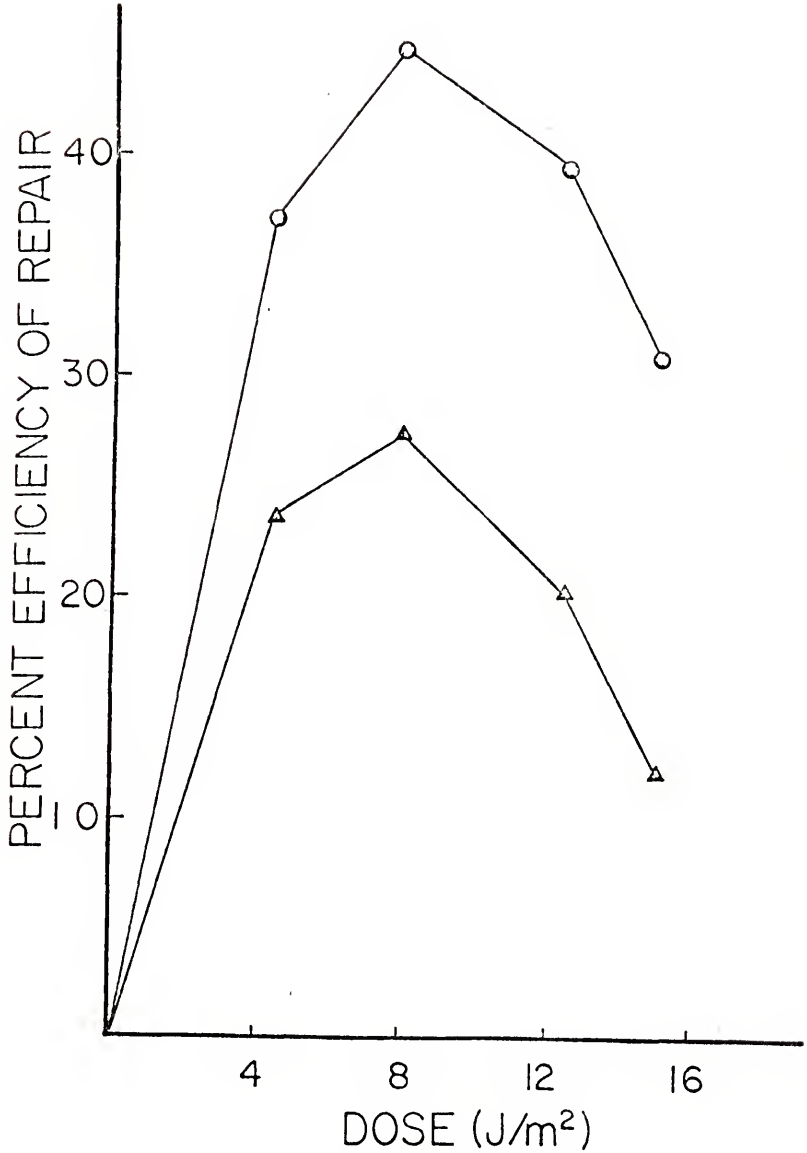

PERCENT EFFICIENCY OF REPAIR
40
30
20
10
4
8
12
16
DOSE (J/m²)

Figure 6. Dose induction kinetics plot for *E. coli* AB2470 *recB*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

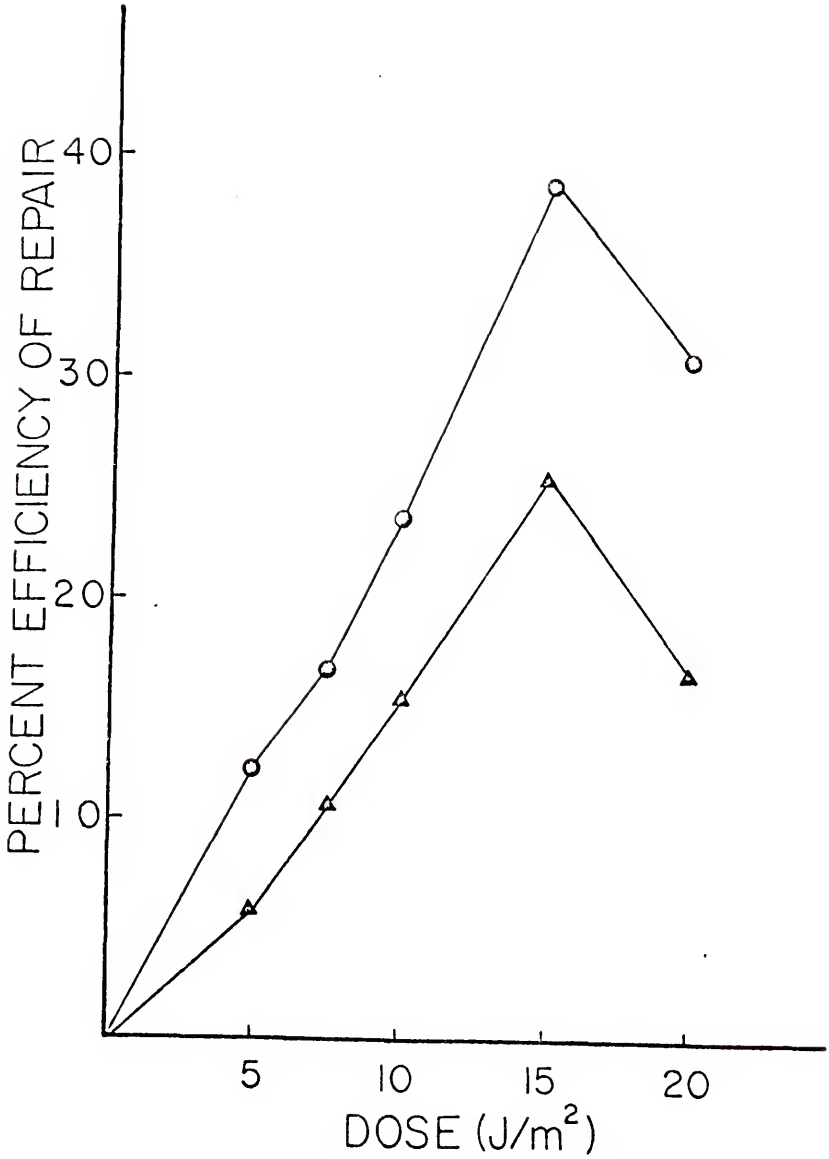

PERCENT EFFICIENCY OF REPAIR
40
30
20
10
5
10
15
20
DOSE (J/m²)

Figure 7. Dose induction kinetics plot for *E. coli* JC5519 *recB* *recC*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

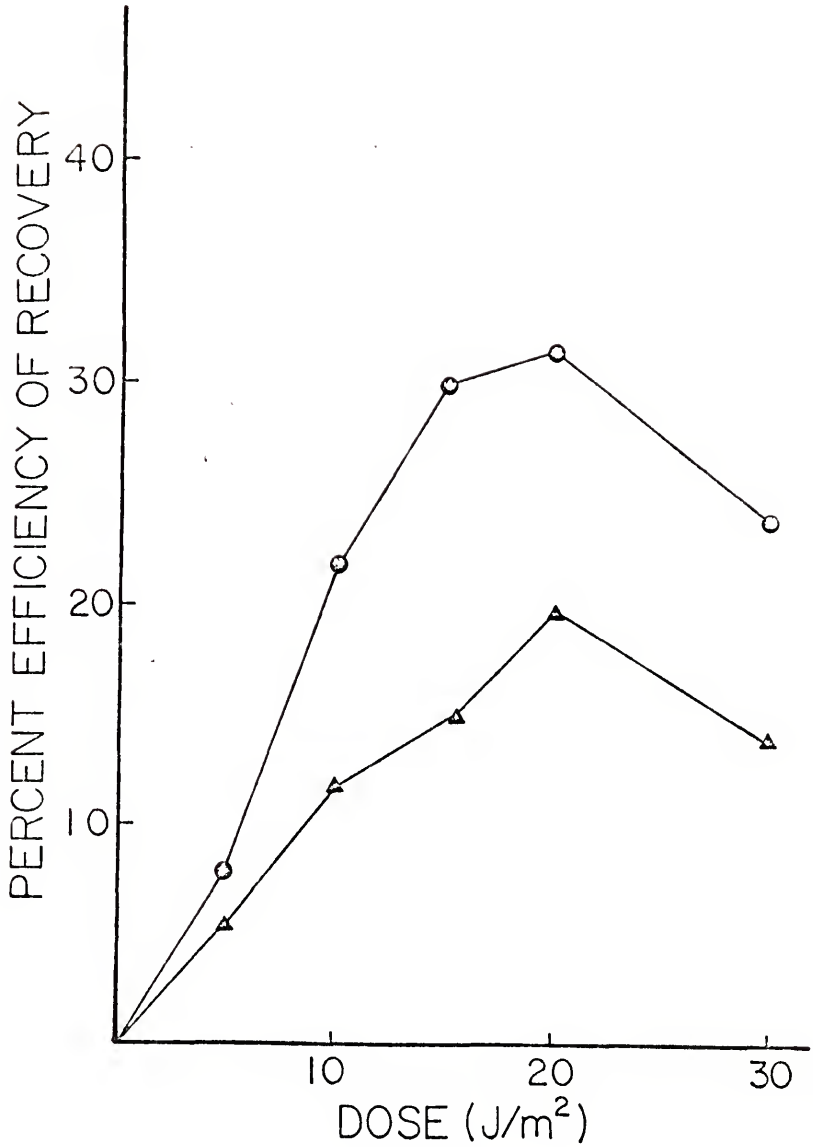

PERCENT EFFICIENCY OF RECOVERY
40
30
20
10
10
20
30
DOSE (J/m²)

Figure 8. Dose induction kinetics plot for *E. coli* AB2463 *recA*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

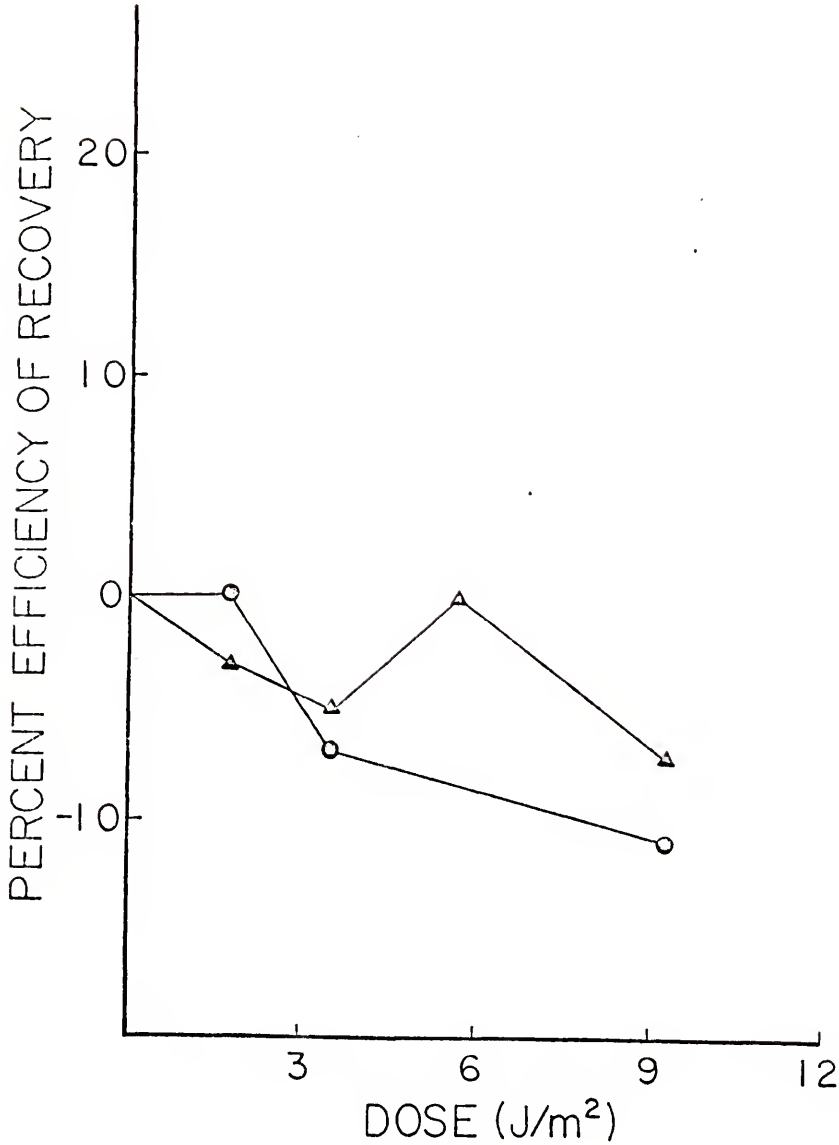

PERCENT EFFICIENCY OF RECOVERY
20
10
0
-10
3
6
9
12
DOSE (J/m²)

Figure 9. Dose induction kinetics plot for *E. coli* AB2494 *lexA*. Circles, single stranded ØX174 DNA; triangles, replicative form ØX174 DNA.

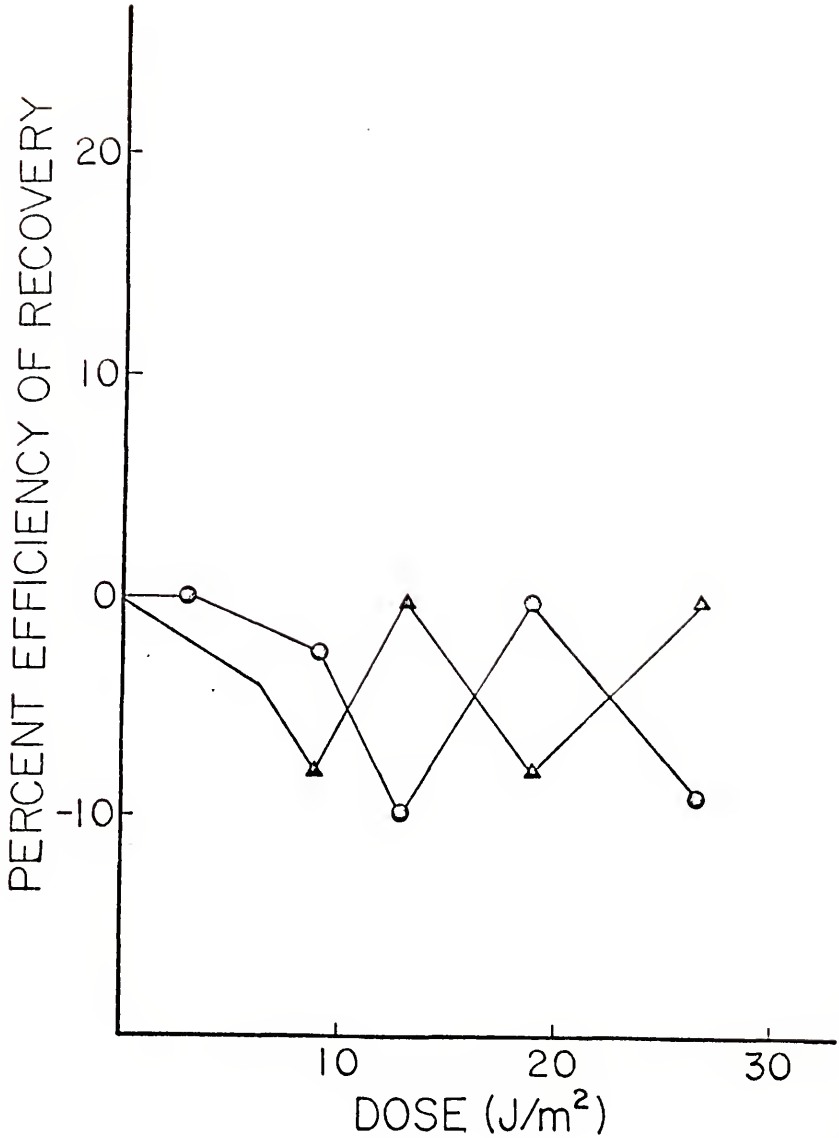

PERCENT EFFICIENCY OF RECOVERY
20
10
0
-10
10
20
30
DOSE (J/m²)

TABLE III

MAXIMUM RECOVERY EFFICIENCIES AND THEIR UV ELECITING DOSES

Host	Dose J/m^2	Efficiency of Recovery		Relative Efficiencies***	
		SS*	RF**	SS	RF
AB1157	70	60.0	45.0	1.0	1.0
AB1186 uvrA⁻	5.0	41.0	27.2	0.68	0.60
AB1184 uvrC⁻	8.0	45.2	27.2	0.75	0.60
AB2470 recB⁻	15.0	39.9	26.6	0.67	0.59
JC5519 recB⁻ recC⁻	20.0	32.2	20.4	0.54	0.45
AB2463 recA⁻	--	0	0	0	0
AB2494 lexA⁻	--	0	0	0	0

*Single stranded ØX174 DNA

**Replicative form ØX174 DNA

***Relative to the wild type strain

the single strand DNA molecule and 45% to 60% of the efficiency of recovery of the replicative form molecule.

There is an order of magnitude difference in the range of the UV inducing doses which give maximum efficiency of recovery. The doses for the different hosts are inversely related to their relative sensitivities to UV. The wild type strain AB1157 is the least sensitive to UV and shows the greatest maximal UV inducing dose while strains AB1886 uvrA and AB1884 uvrC are the most sensitive to UV and show the smallest maximal UV inducing dose. Strains A2470 recB and JC5519 recB recC have intermediate UV sensitives and reflect this in their maximal UV inducing doses.

Table IV lists the efficiencies of UV induced recovery for single stranded and replicative form ØX174 DNA in three hosts all lacking a functional correndonuclease II and are all defective in the incision step of excision repair. Two of these hosts, AB1886 and AB1889, are mutant at the uvrA gene but at different loci while the third host, AB1885, is mutant at the uvrB gene. Little difference is seen in the efficiencies of UV induced recovery in these three hosts at the two UV inducing doses used.

UV Induced Recovery of Gamma Irradiated DNA

Figures 10 through 17 show the plots of percent survival of phage producing ability versus gamma ray dose for single stranded and replicative form ØX174 DNA when UV induced or uninduced E. coli AB1157 were the host. The UV inducing dose is 70 J/m^2. In all instances there is greater survival of phage producing ability with the UV induced host than the uninduced host. Table V summarizes the efficiencies of UV induced repair observed under the various radiation conditions employed.

TABLE IV

EFFICIENCY OF UV INDUCED REPAIR OF ØX174 DNA
IN THREE EXCISION REPAIR DEFECTIVE MUTANTS

Dose (J/m^2) To Host	Single Stranded ØX174		
	1885 uvrB5	1886 uvrA6	1889 uvrA35
2.5	38.9	38.2	35.8
7.5	36.2	33.6	44.3

Dose (J/m^2) To Host	Replicative Form ØX174		
	1885 uvrB5	1886 uvrA6	1889 uvrA35
2.5	30.0	23.9	22.4
7.5	22.7	21.2	20.8

Figure 10. Survival of the phage producing ability of gamma irradiated ØX174 single stranded DNA bubbled with nitrogen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

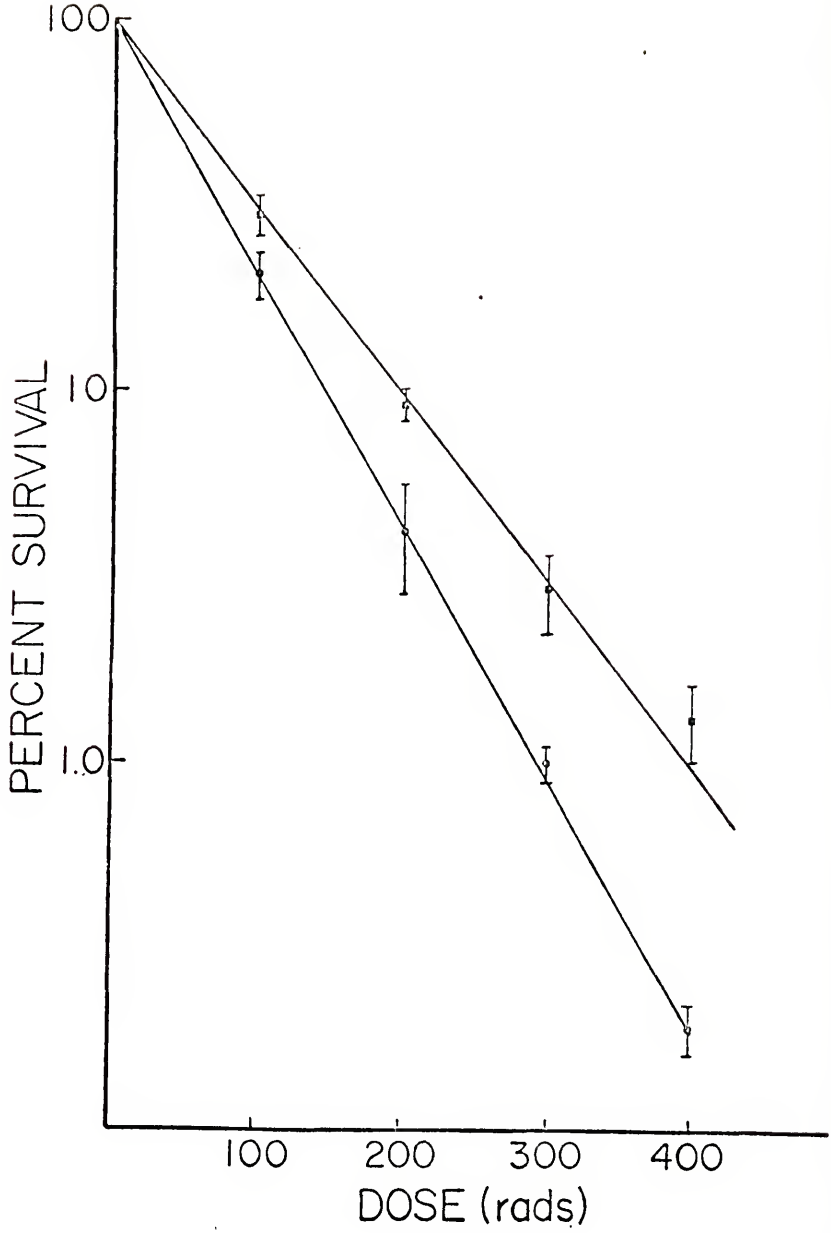

100
10
1.0
PERCENT SURVIVAL
100
200
300
400
DOSE (rads)

Figure 11. Survival of the phage producing ability of gamma irradiated ØX174 replicative form DNA bubble with nitrogen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli as host.

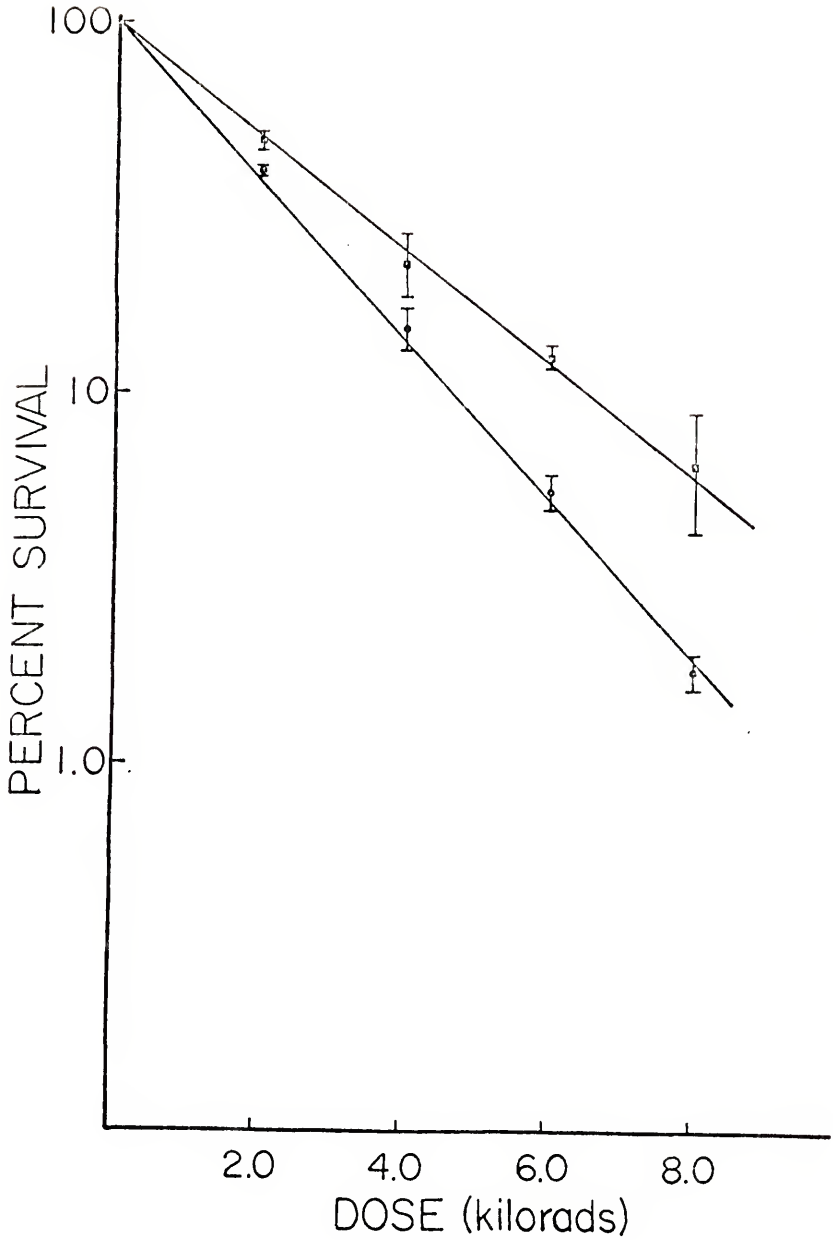

100
10
1.0
PERCENT SURVIVAL
2.0
4.0
6.0
8.0
DOSE (kilorads)

Figure 12. Survival of the phage producing ability of gamma irradiated ØX174 single stranded DNA bubbled with oxygen. Circles, unirradiated *E. coli* AB1157 as host; squares, UV irradiated (70 J/m^2) *E. coli* AB1157 as host.

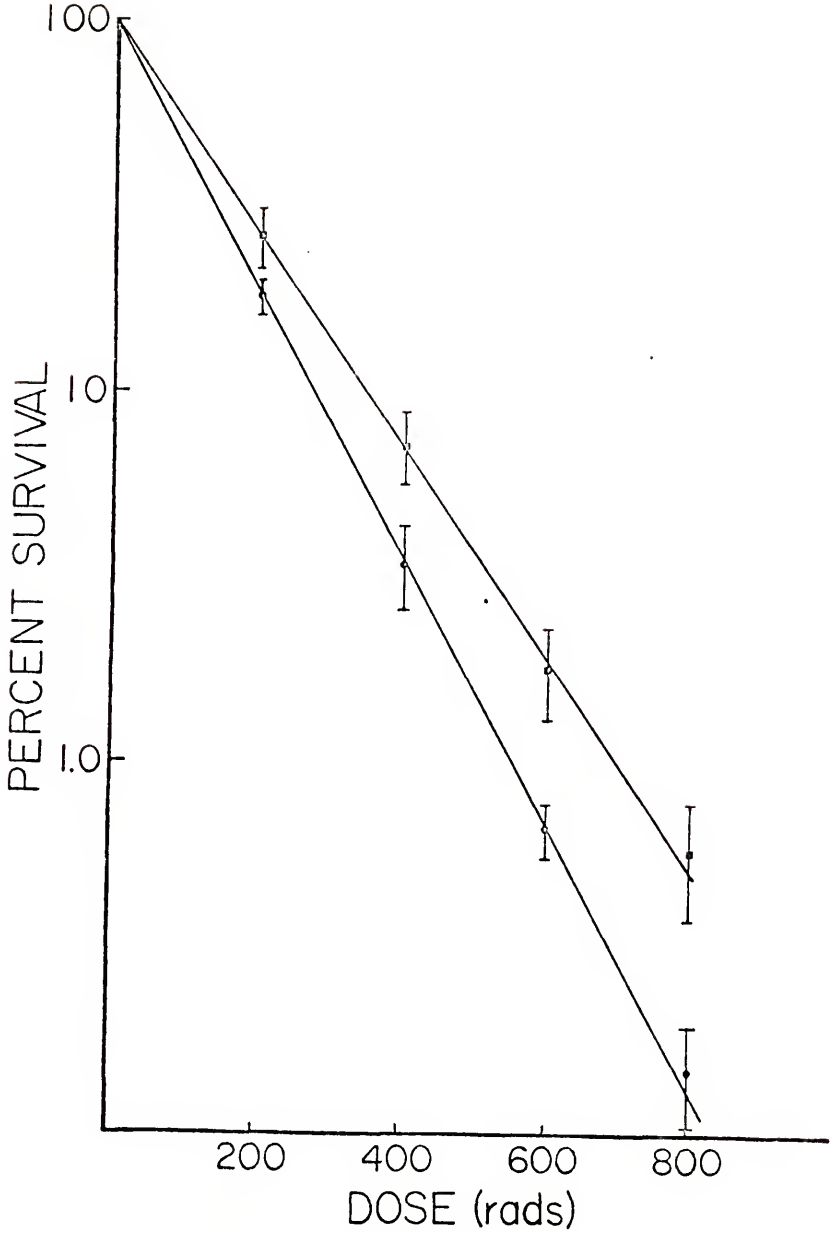

100
10
1.0
PERCENT SURVIVAL
200
400
600
800
DOSE (rads)

Figure 13. Survival of the phage producing ability of gamma irradiated ØX174 replicative form DNA bubbled with oxygen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

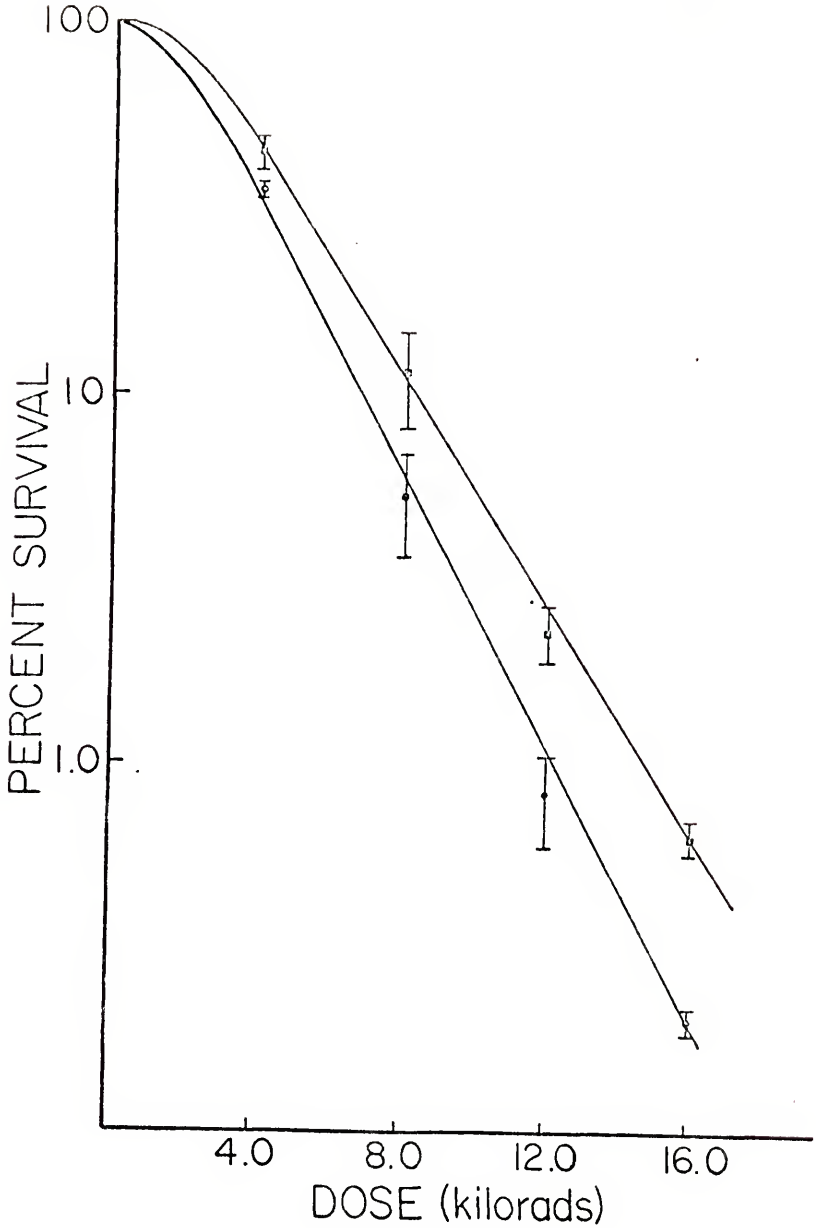

100
10
1.0
PERCENT SURVIVAL
4.0
8.0
12.0
16.0
DOSE (kilorads)

Figure 14. Survival of the phage producing ability of gamma irradiated ØX174 single stranded DNA in the presence of 0.05 M potassium iodide and bubbled with nitrogen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

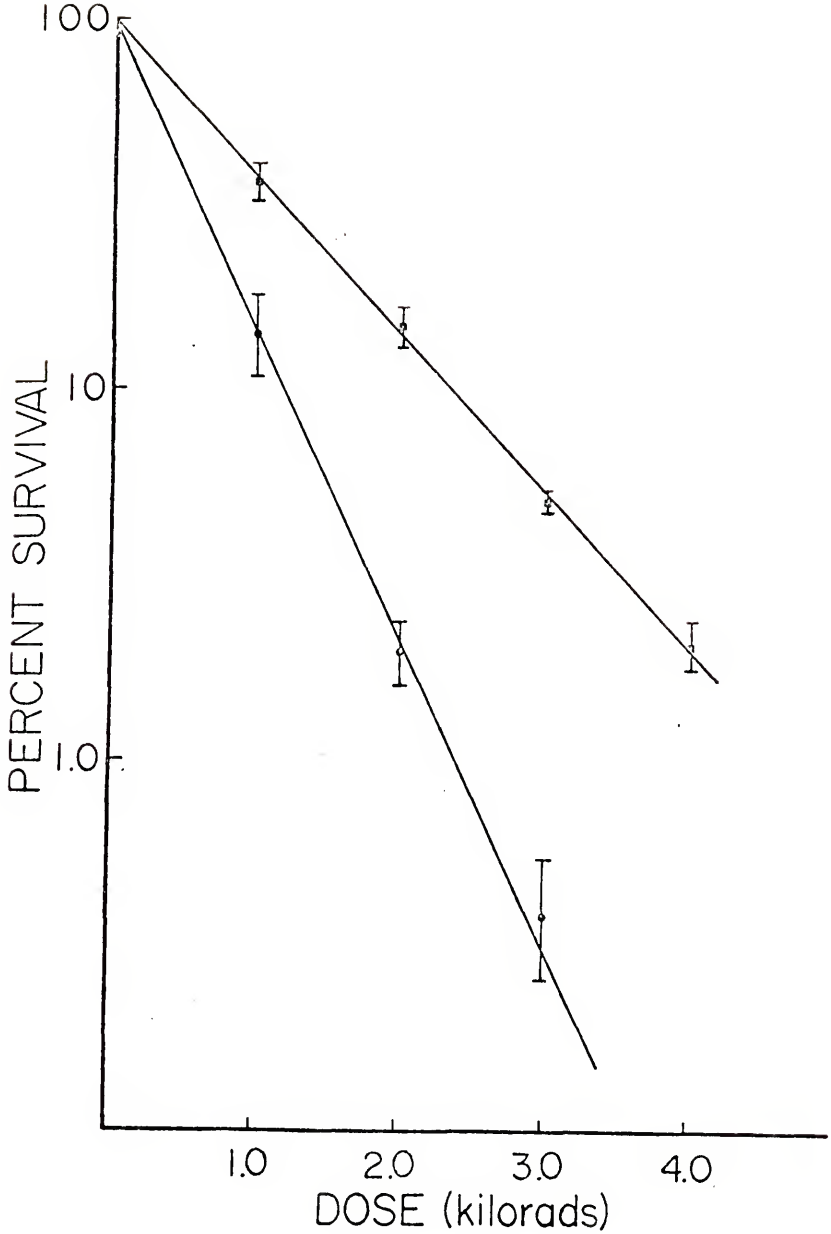

100
10
1.0
PERCENT SURVIVAL
1.0
2.0
3.0
4.0
DOSE (kilorads)

Figure 15. Survival of phage producing ability of gamma irradiated ØX174 replicative form DNA in the presence of 0.05 M potassium iodide and bubbled with nitrogen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

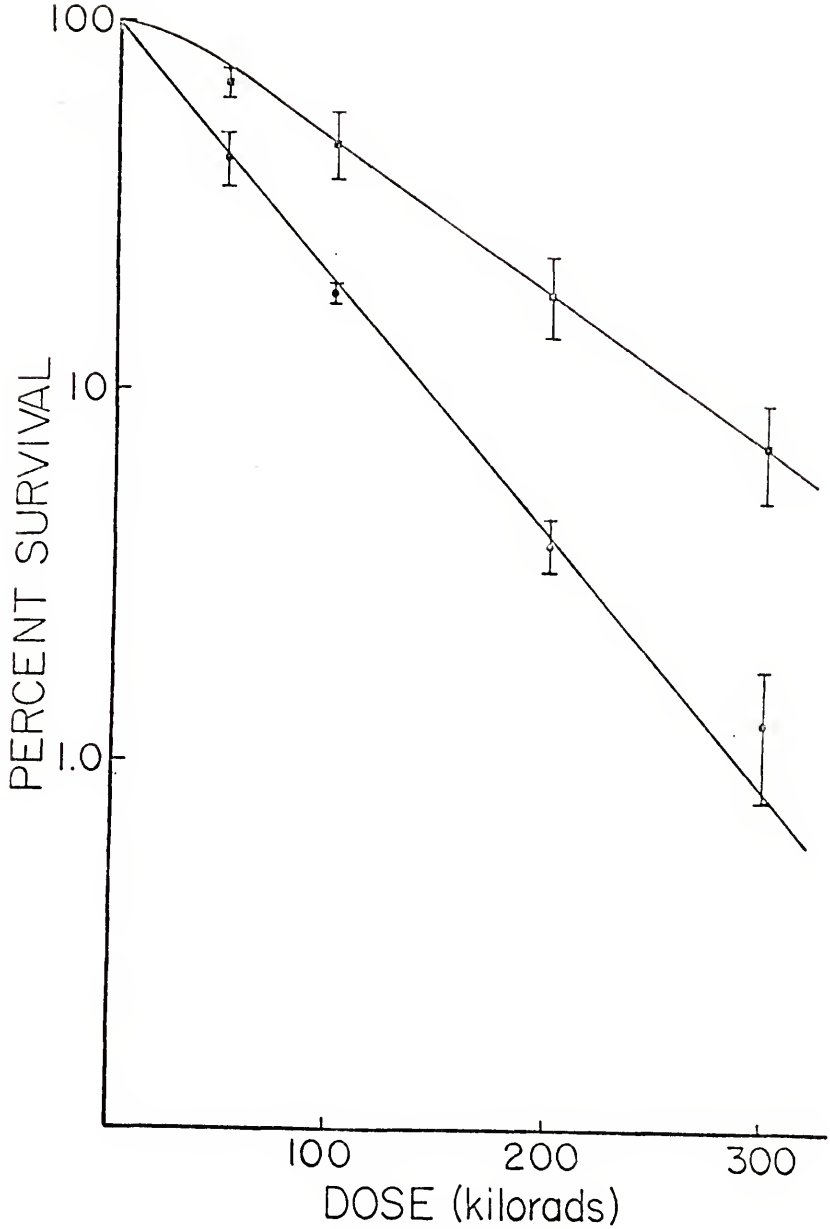

100
10
1.0
PERCENT SURVIVAL
100
200
300
DOSE (kilorads)

Figure 16. Survival of phage producing ability of gamma irradiated ØX174 single stranded ØX174 DNA in the presence of 0.05 M potassium iodide and bubbled with oxygen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

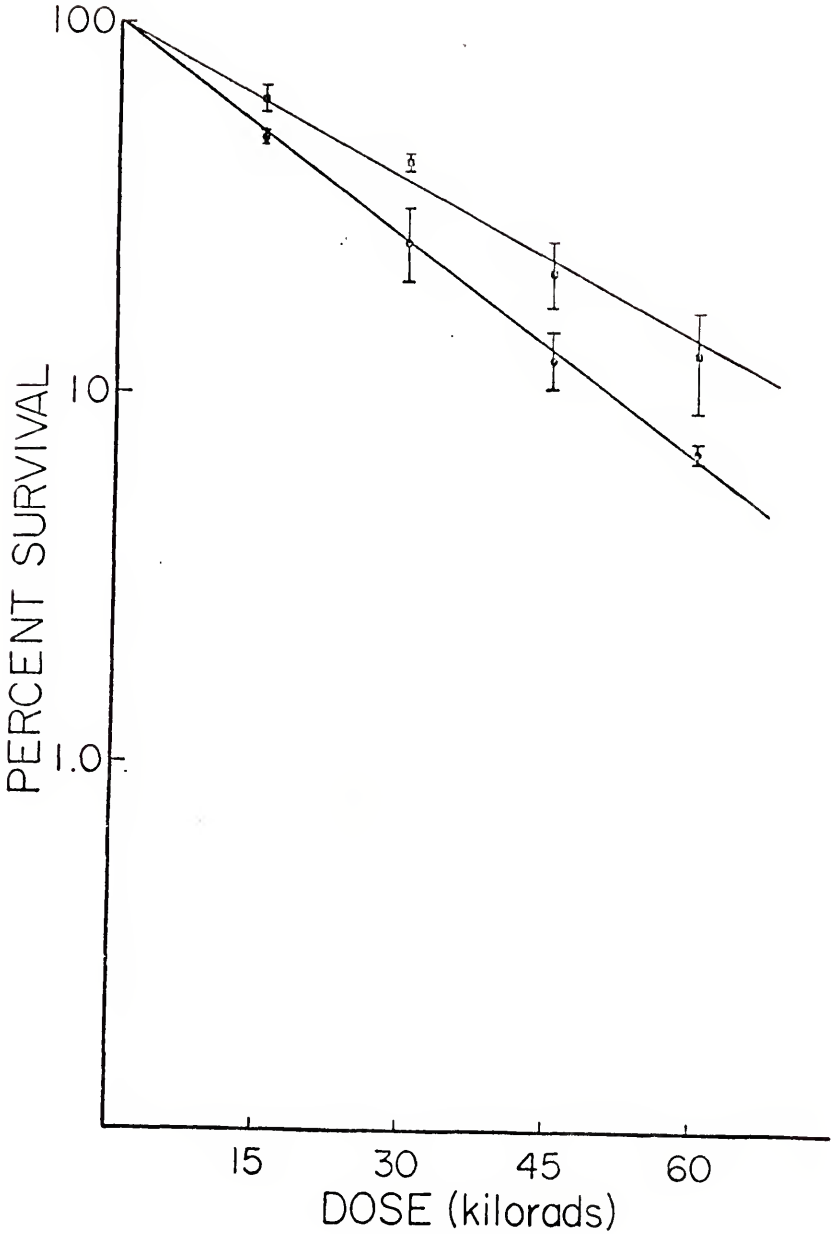

100
10
1.0
PERCENT SURVIVAL
15
30
45
60
DOSE (kilorads)

Figure 17. Survival of phage producing ability of gamma irradiated ØX174 replicative form DNA in the presence of 0.05 M potassium iodide and bubbled with oxygen. Circles, unirradiated E. coli AB1157 as host; squares, UV irradiated (70 J/m^2) E. coli AB1157 as host.

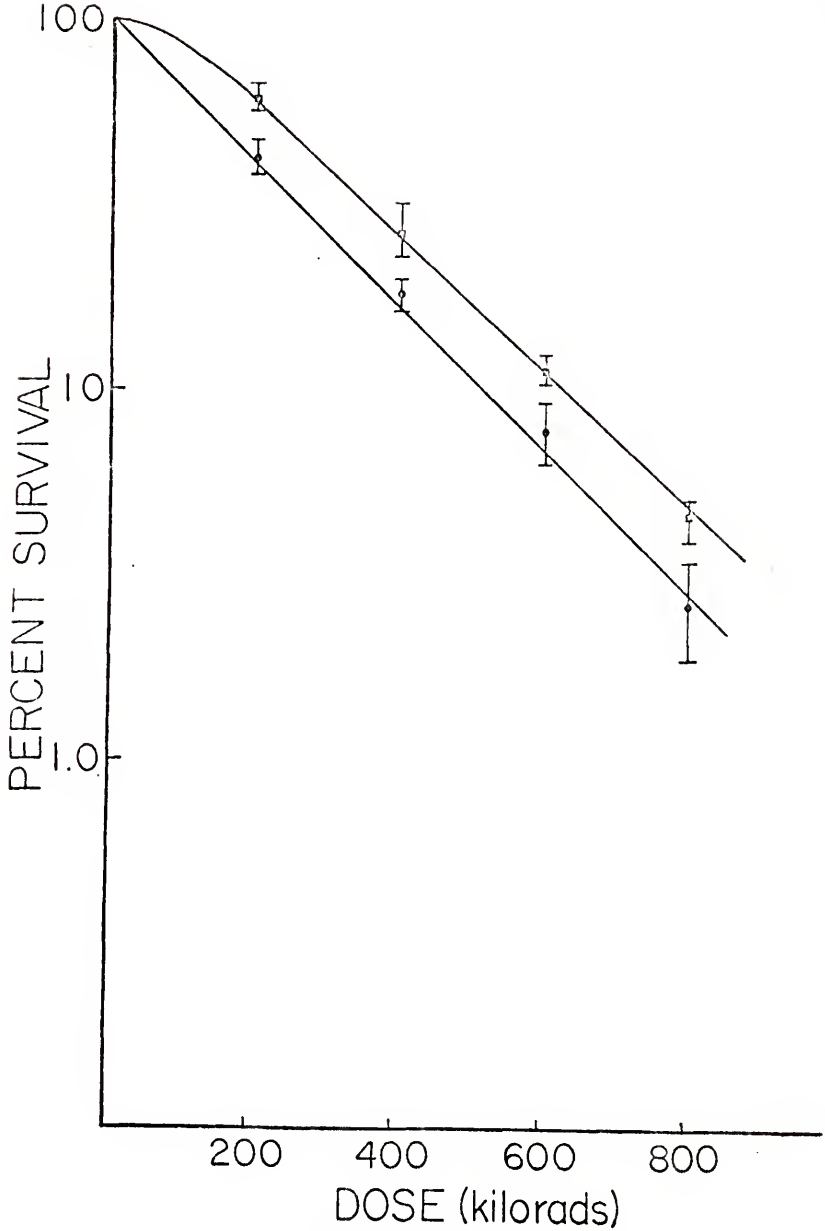

100
10
1.0
PERCENT SURVIVAL
200
400
600
800
DOSE (kilorads)

TABLE V

EFFICIENCIES OF UV INDUCED RECOVERY OF ØX174 DNA
GAMMA IRRADIATED UNDER VARIOUS CONDITIONS

Radical Scavenger	Radicals Reacting with DNA	Efficiency of Recovery	
		SS*	RF**
N_2	Solvated electron Hydrogen radical Hydroxyl radical	25.3	28.6
O_2	Hydroxyl radical	20.8	19.9
I-	Solvated Electron Hydrogen radical	48.7	46.5
I- and O_2	None (direct action)	22.4	20.0

*Single stranded ØX174 DNA

**Replicative form ØX174 DNA

Several observations are apparent from Table V. First, for a given radiation condition the efficiency of UV induced recovery is the same for both types of ØX174 DNA molecules. Secondly, radiation conditions which effectively scavenge the hydroxyl radical produce the largest efficiency of recovery. Thirdly, the efficiencies of recovery that result from no radical attack on the DNA (direct action) or hydroxyl radical attack alone are the same. Conditions which do not scavenge any of the radicals result in efficiencies of recovery which are only slightly higher than those of direct action or hydroxyl radical attack.

DISCUSSION

The purpose of this dissertation was to elucidate some of the properties of UV induced recovery in E. coli of UV and gamma irradiated single and double stranded DNA molecules. Five objectives were set forth in the Introduction as a framework for doing this. What follows is a discussion of how this study meets these objectives.

Figures 3 through 7 and Table IV show that in every strain in which UV induced recovery of UV damaged DNA occurred, the efficiency of recovery of the single stranded ØX174 DNA molecule is more than that of the double stranded replicative form molecule. There are several possible explanations that could account for this difference. One could be that when both types of molecules are given UV doses which will reduce their phage producing capacities to the same level, the replicative form molecule will contain more damage than the single stranded molecule. This greater amount of damage presumably could inhibit the UV induced recovery mechanism from restoring the molecule to a biologically active state. The yield of pyrimidine dimers in both single stranded and replicative form ØX174 DNA is known (20, 37). The number of dimers present after both types of DNA molecules have been given a UV dose which results in equal phage producing ability in an induced host can be computed. The host chosen for this calculation must possess only the UV induced recovery system as its only method of repairing UV damaged ØX174 DNA. The data in Table VI must be consulted to choose such a host.

TABLE VI

UV SENSITIVITIES OF SINGLE STRANDED AND REPLICATIVE FORM ØX174 DNA MOLECULES IN VARIOUS UNIRRADIATED HOSTS

Strain	D_{37} (J/m^2)	
	Single Strand	Replicative Form
AB1157 wild type	8.5	75
AB1184 uvrC⁻	8.5	16
AB1885 uvrB⁻	9.1	15
AB1886 uvrA⁻	9.1	13
AB1889 uvrA⁻	8.5	16
AB2463 recA⁻	8.0	70
AB2470 recB⁻	9.0	70
JC5519 recB⁻ recC⁻	8.3	70

Table VI lists the D_{37} doses determined in this study for the inactivation of single stranded and replicative form ØX174 DNA in unirradiated hosts. The D_{37} dose is that dose which on the average causes one lethal event for every DNA molecule in the irradiated population. It is a measure of the UV sensitivity of the ØX174 DNA in a particular host and a measure of the repair capability offered by that host. The higher the D_{37} dose, the greater the recovery from UV damage. Table VI shows that the sensitivity of the single stranded ØX174 DNA is the same in all unirradiated hosts indicating that excision repair and recombination do not participate in the recovery of this molecule from UV damage. On the other hand, the replicative form molecule is more sensitive in excision repair deficient hosts than in the wild type or recombination deficient strains. This indicates that excision repair plays a role in the recovery of the UV irradiated replicative form molecule. The wild type and recombination deficient hosts, however, have the same UV sensitivities indicating that recombination does not contribute to the recovery of the replicative form molecule. Thus, the only recovery mechanism available in excision repair deficient mutants for single stranded and replicative form ØX174 DNA is the UV induced recovery mechanism.

Using UV irradiated excision repair deficient strains, Table VII shows the number of dimers calculated to be present in single stranded and replicative form molecules which have been exposed to a D_{37} dose of UV. The number of dimers is approximately the same. Thus, the difference in single stranded and replicative form ØX174 DNA UV induced recovery cannot be explained by different amounts of UV damage in each type of molecule.

TABLE VII

DIMER YIELDS AT D_{37} FOR SINGLE STRANDED AND REPLICATIVE FORM ØX174 DNA IN EXCISION REPAIR DEFICIENT HOSTS

Strain*	Number of Dimers**	
	Single Stranded	Replicative Form
1885 uvrB-	1.1	1.2
1886 uvrA-	1.0	1.0
1889 uvrA-	1.0	1.1

*The hosts have been irradiated with 2.5 J/m^2 of UV

**Number of dimers = D_{37} dose x dimer yield

Another possibility could be that the action of UV induced recovery could produce configurations of the replicative form molecule which are susceptible to nuclease attack. Degradation or a double strand break would result in the inactivation of the DNA molecule.

A third possibility is to assume that the single stranded ØX174 DNA molecule is a better substrate for UV induced recovery than the replicative form. This explanation is particularly attractive if it is assumed that a polymerase like that of SOS repair mediates in the UV induced recovery of ØX174 DNA. Such a polymerase might be better suited for asymmetric rather than semi-conservative DNA replication for a variety of reasons. Steric hindrance by one strand of the replicative form molecule could inhibit the ability of the polymerase to use the other strand as a template for DNA synthesis. Also, the polymerase may lack the ability to interact properly with other proteins which normally play a part in DNA replication. This could result in a lowering of the activity of a replication associated function, such as the ability to unwind duplex DNA, that is necessary for the efficient use of double stranded DNA molecules as a template. The net result of this or of any of the other possible explanations described in the above paragraphs would be to lower the efficiency of UV induced recovery for the replicative form molecule.

The results of this study show that the UV induced recovery of UV irradiated single stranded and replicative form ØX174 DNA does not depend upon the uvrA, uvrB, uvrC, recB and recC gene products but does depend upon the recA and lexA gene products. This indicates that the UV induced recovery of ØX174 DNA like the UV induced recovery of other bacteriophage and bacterial systems proceeds by a mechanism

independent of excision repair and recombination mediated by the recB recC pathway (22, 94). Examination of Table III, however, shows that the levels of UV induced recovery in the excision repair and recombination deficient strains are anywhere from 75% to 45% of the efficiency of recovery for the wild type host. This indicates that it is possible to modulate the level of UV induced recovery. The best explanation of the disparity in the levels of efficiency of recovery is to assume that the missing gene products play a role in producing the inducing signal which ultimately turns on the UV induced recovery mechanism. It has been suggested that the inducing signal in UV irradiated E. coli may be the products resulting from the repair of UV damage to the bacterial DNA (94). The uvrA, uvrB, uvrC, recB and recC gene products all play a role in the metabolism of UV irradiated bacterial DNA. It might be that all of the DNA degradation products produced by the action of these gene products act as the inducing signal in some cooperative manner. Removing one set of these products could lessen the likelihood of a successful induction event. This would result in fewer induced host cells in the irradiated population which in turn would result in a lowering of the efficiency of UV induced recovery with respect to the wild type host.

That the differences in the efficiencies of UV induced recovery are not due merely to metabolic or physiological peculiarities among the various uvr strains used in these experiments is demonstrated in Table IV. All three strains share a common defect, the inability to perform the incision step of excision repair. Two of the defects map in separate loci in the uvrA gene while the other is in the uvrB gene. In all three strains, however, the efficiencies of UV induced

recovery for both forms of ØX174 DNA at two different UV inducing doses to the host are essentially the same. This indicates that the level of efficiency of recovery is dependent upon a shared step in the metabolism of UV irradiated bacterial DNA.

The wide range of UV doses required to elicit maximum efficiency of recovery for ØX174 DNA in the various hosts shown in Table III is also observed in other systems (22, 94). Witkin explains this variation by assuming that pyrimidine dimers must persist in bacterial DNA if induction is to occur (94). It is then assumed that repair proficient cells can effectively remove dimers from their DNA before the inducing signal can be generated. Thus, a large UV dose is required to insure that at least one dimer will escape being repaired long enough so that it can participate in the induction process. For cells less proficient in repairing dimers, a smaller dose is required to ensure induction. Therefore, the most repair competent strain AB1157 has the highest UV induction dose while the least repair competent strain AB1886 uvrA- has the lowest UV induction dose. The strains AB2470 recB- and JC5519 recB- recC- are intermediate in their UV repair capabilities and reflect this in their intermediate UV inducing doses (39, 91).

Table V compares the efficiency of UV induced repair which resulted from the transfection of E. coli AB1157 irradiated with 70 J/m^2 of UV with gamma irradiated ØX174 DNA. For all irradiation conditions, UV induced recovery of both types of DNA molecules is observed. The level of the efficiency of recovery, however, is dependent upon what water radiolysis radicals are attacking the DNA. The greatest amount of recovery is seen when the hydrogen radical and solvated electron

are the damaging species and the smallest amount of recovery is seen when the DNA is damaged either by the hydroxyl radical alone or by direct action. The results tabulated in Table V also suggest that each radiation condition may produce two sets of damage. One set of damage is susceptible to UV induced recovery while the second set is not. The fraction of total damage susceptible to UV induced recovery in the population of irradiated DNA molecules would be equal to the efficiency of recovery for a given radiation condition. If this assumption is true, Table V suggests that the yields of both types of these damages are equal in both types of ØX174 DNA.

The argument for susceptible and non-susceptible classes of damage is further supported by the fact that when the action of the hydroxyl radical is added to that of the hydrogen radical and solvated electron, the efficiency of UV induced recovery is severely reduced. This suggests that the hydroxyl radical can produce damage which masks the recovery from damage caused by the other two radical species.

The chemical nature of the susceptible and non-susceptible classes of gamma ray induced damage in ØX174 DNA cannot be identified by this study. Some inferences can be made about the nature of the susceptible gamma ray damage in both types of the ØX174 DNA molecules, however. It is most likely that this type of damage is base damage rather than strand breaks. A single strand break converts the covalently closed, single stranded ØX174 DNA molecule into a linear one. The linear molecule has no biological activity. Unless the UV induced recovery mechanism can convert a single stranded, linear molecule back to a covalently closed one, an activity which has never been reported, some variety of base damage probably constitutes

the susceptible lesion. The same may be true of the replicative form molecule since single and double strand breaks only contribute to 10% of gamma ray lethality (87). If base damage is indeed the susceptible damage, then all radiation conditions produce this damage to a greater or lesser degree.

The levels of UV induced recovery of gamma irradiated ØX174 DNA are the same for both single stranded and replicative form molecules. This is in contrast with the efficiency of UV induced repair being higher for irradiated single stranded molecules than for UV irradiated replicative form molecules. This indicates that gamma irradiated ØX174 DNA is an equally good substrate for UV induced recovery regardless of whether the molecule is single or double stranded. Damage such as UV induced pyrimidine dimers cause greater helix distortion in duplex DNA molecules than the single strand breaks and base damage caused by gamma rays (17). The lack of such distortion in a UV irradiated single stranded molecule may account for its greater recovery than that found for the UV irradiated replicative form. It does not explain why UV irradiated single stranded DNA shows a higher efficiency of recovery than its gamma irradiated counterpart. Apparently other characteristics of the radiation induced damages different from helix distortions account for these particular responses of the two forms of DNA molecules to the damage produced by the two types of radiation.

In many respects the UV induced recovery observed in this study is similar to the W-reactivation of single and double stranded bacteriophage (21, 22, 36, 49, 58, 83, 90). Here irradiated, purified DNA is used to transfect UV irradiated, calcium treated E. coli

rather than irradiated bacteriophage infecting irradiated, whole bacteria. The phage and DNA show a greater recovery from the lethal effects of radiation when they infect or transfect UV irradiated hosts. Another similarity between the UV induced recovery of irradiated ØX174 DNA and W-reactivation is their common requirement for the recA and lexA gene products. Also, both processes are mediated by a recovery mechanism which is independent of excision repair and recombination since both occur in excision repair defective and recombination defective (other than recA) mutants. Based on these similarities, the UV induced recovery of irradiated ØX174 single and double stranded DNA and W-reactivation are essentially the same.

Biological and in vitro biochemical evidence indicate that W-reactivation of single stranded DNA occurs by the conversion of the UV damaged, single stranded molecule to a full length, covalently closed replicative form (60, 61, 94). This replicative form is necessary if whole phage are to be produced (50). Radman's proposed SOS repair mechanism which involves a hypothetical polymerase capable of effecting such a conversion could very nicely account for single stranded bacteriophage W-reactivation. Such a mechanism could also account for the UV induced recovery of UV irradiated ØX174 single stranded DNA.

The UV induced recovery of UV irradiated replicative form ØX174 DNA can also be explained by the same mechanism. The sequence of ØX174 DNA replication after the formation of the parental replicative form leads to the production of daughter replicative form molecules which in turn serve as templates for the production of progeny single

stranded DNA molecules (27, 28, 70). All of the DNA synthesis in these steps is asymmetrical. A pyrimidine dimer in the complementary strand of the replicative form could block any further DNA replication since this strand is the primary template for daughter replicative form and progeny single stranded DNA synthesis. UV damage in the complementary strand which blocked DNA replication would render the replicative form biologically inactive. The hypothetical SOS repair polymerase would be able to use the damaged viral strand as a template for further DNA replication. Thus, this mechanism could restore the biological activity of the UV damaged replicative form molecule.

From the data in Table VI, the yield of dimers at D_{37} in the single stranded and replicative form ØX174 DNA molecules in an unirradiated excision repair deficient host can be calculated. They are 0.75 and 0.88 respectively. These numbers are approximately equal to 1.0, the expected number if only one dimer were required in the single stranded DNA molecule or in the template strand of the replicative form molecule to inactivate it. This represents evidence supporting the contention that a dimer in the complementary strand of the replicative form molecule is a lethal event. In turn this supports the speculation that the same mechanism which mediates in the UV induced recovery of single stranded ØX174 DNA could participate in the recovery of the replicative form molecule.

The recovery of gamma ray damaged ØX174 DNA could be carried out by the same mechanism described above for the UV irradiated molecules if it is assumed that gamma ray damage can block the asymmetric DNA replication necessary for a single stranded or

replicative form ØX174 DNA molecule ultimately to produced whole phage. Whether or not gamma ray damage can block bacteriophage DNA replication is not known. Since UV irradiation of the host bacteria results in the greater recovery of the biological activity of both gamma and UV irradiated ØX174 DNA, it is attractive to assume that the UV induced recovery of both types of damage is mediated by a common system. However, the differences in the chemical structures, the repair and the biological consequences of known UV and gamma ray DNA damages suggest that it is very possible that the UV induced recovery of gamma irradiated ØX174 DNA could occur by a completely different mechanism than that which effects the recovery of UV irradiated ØX174 DNA. Whether or not UV induced recovery of gamma irradiated ØX174 DNA can be carried out by a SOS repair type of polymerase cannot be surmised until the effects of gamma ray damage on bacteriophage DNA replication have been determined.

To summarize, this study demonstrates that both single and double stranded ØX174 DNA damaged by either UV or gamma radiation is susceptible to UV inducible recovery. The results presented suggest that the amount of inducible recovery is dependent upon the type of damage introduced into the DNA molecules and whether the molecule is single or double stranded. Also, the data shown indicate that the level of expression of the inducible recovery system can be modulated by the action of gene products normally involved in DNA repair. It can be surmised that the inducible recovery of both types of DNA whether UV or gamma irradiated is mediated by a common mechanism possibly that proposed by SOS repair. The results of this study do not unambiguously support this conjecture which can only be verified by further lines of experimentation.

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BIOGRAPHICAL SKETCH

John Randall Silber was born January 10, 1949 in Philadelphia, Pennsylvania. In 1954 he moved to St. Petersburg, Florida, where he graduated from Northeast High School in June of 1967. Attending the University of Florida, he received a Bachelor of Science degree in physics in December 1971. He returned to the University of Florida in September of 1973 where he is currently a doctoral candidate in the Department of Microbiology and Cell Science.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Doctor of Philosophy.

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